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Descriptions of four new dextral land snails of the genus *Camaena* (Gastropoda, Eupulmonata, Camaenidae) from south China

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Abstract

In this study, four new dextral camaenid from China are reported, based on shell morphology, reproductive system anatomy, and molecular phylogenetic analyses: *Camaena funingensis* Zhou, Wang & Lin, **sp. nov.**, *Camaena gaolongensis* Zhou, Wang & Lin, **sp. nov.**, *Camaena maguanensis* Zhou, Wang & Hu, **sp. nov.**, and *Camaena yulinensis* Zhou, Wang & Hu, **sp. nov.** Detailed descriptions of the morphological characteristics including shells and genitalia, DNA sequences, and living environments of the four new species are provided, with further comparisons with congeners.

Keywords

Anatomy, *Camaena*, molecular biology, shell morphology, terrestrial snail

Introduction

The genus *Camaena* was established by Albers (1850). It is the speciose type genus in the family Camaenidae, with the type species *Helix cicatricosa* Müller, 1774. The species in this genus are mainly distributed throughout southern China, Indochina, and

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beyond in Southeast Asia, and most are locally endemic (Pilsbry 1894; Zilch 1959–1960, 1964; Richardson 1985; Chen and Gao 1987; Ding et al. 2016; Inkhavilay et al. 2019). The genus was divided into five subgenera (*Camaena* Albers, 1850, *Camaenella* Pilsbry, 1893, *Pseudobba* Moellendorff, 1891, *Pancala* Kuroda & Habe, 1949, *Miyakoia* Minato, 1980) on the basis of classifications by Pilsbry (1894), Kuroda and Habe (1949), Zilch (1959–1960), and Vaught (1989). A recent molecular phylogeny (Hoso et al. 2010) and anatomical study (Hwang 2012) suggested that *Pancala* and *Miyakoia* should be synonyms of the confamilial genus *Satsuma*.

There are 24 species of the genus distributed in southern China belonging to two subgenera, *Camaena* and *Camaenella*. Twenty-three species belong to *Camaena* (Yen 1939; Chen and Zhang 1999; Schileyko 2003; Ai et al. 2016; Ding et al. 2016), and only one species is in *Camaenella* (Pilsbry 1894; Yen 1939; Chen and Zhang 1999). The subgenus *Camaenella* was treated as a synonym of *Camaena* or as a genus in its own right by some scholars (Chen and Gao 1987; Chen and Zhang 1999). In this article, *Camaenella* will be considered as a valid subgenus.

Camaena species are divided into a sinistral group and a dextral one. They are usually characterized by a moderately solid shell with scar-like protrusions or malleations, 4.5–5.5 slightly convex whorls, a brown or yellow surface with red or puce spiral bands, and reflexed aperture margins (Schileyko 2003; Ai et al. 2016). The classification of *Camaena* has mainly relied on the shell features. Anatomical and molecular studies of *Camaena* are rare, except for the sinistral and the newly described species (Chen and Zhang 1999; Ai et al. 2016; Ding et al. 2016; Páll-Gergely et al. 2016; Wu et al. 2019). Historically, the classification of this genus is rather confused. For the sinistral group, the taxonomic status has always been controversial, and scientific names have been revised repeatedly. Ding et al. (2016) revised *C. cicatricosa* as four species, *C. cicatricosa*, *C. inflata* (Möllendorff, 1885), *C. obtecta* (Fischer, 1898), and *C. connectens* (Dautzenberg & Fischer, 1906), and described one new species *C. poyuensis* Zhou, Wang & Ding, 2016 using morphological and molecular studies. In the same year, Ai et al. (2016) described two new species *C. lingyunensis* Zhou & Lin, 2016 and *C. detianensis* Zhou & Lin, 2016 according to shell morphology, reproductive system and molecular biology. Thus, the sinistral *Camaena* group contains 12 species or subspecies to date (Schileyko 2003; Ai et al. 2016; Ding et al. 2016). The dextral group can be divided into three informal subgeneric groups according to the morphological characteristics of the shell, especially the shape and location of the carina.

1. Group I possesses an acute and moderate carina on the body whorl. This group could be further divided into two categories by shell height i.e., a relatively low and flat spire, which includes *C. longsonensis* (Morlet, 1891), *C. jinpingensis* Chen, Zhang & Li, 1990 and *C. vorvonga* (Bavay & Dautzenberg, 1900); a relatively high spire, e.g., *C. vayssierei* (Bavay & Dautzenberg, 1909).
2. Group II possesses a blunt carina, which is placed on the higher or middle parts of the body whorl, such as *C. vulpis* (Gredler, 1887), *C. leonhardti* (Möllendorff, 1888), and *C. choboensis* (Mabille, 1889).

3. Group III possesses a smooth periphery, e.g., *C. hainanensis* (Adams, 1870) and *C. xanthoderma* (Möllendorff, 1882).

In this study, the authors have examined many specimens collected in Guangxi and Yunnan in southern China between 2013 and 2015, and discovered four new dextral species on the basis of morphological, anatomical, and molecular evidence, and living environments.

Materials and methods

Specimens were collected by the authors from several sites in China (Fig. 1). The longitude and latitude were recorded using a GPS. The map was established by MapInfo Professional 15.0. The live adults were drowned in water for 12–24 hours, and then killed in hot water. Soft bodies were preserved in 95% ethanol and stored at -20 °C. Empty shells were cleaned and preserved at room temperature in the Key Laboratory of Molluscan Quarantine and Identification of Fuzhou Customs District, Fujian, China (**GACC**).

Shells were measured to 0.1 mm using electronic calipers. Standard shell parameters were taken following Dillon (1984). All adult specimens of each species were measured. Live sexually mature specimens were dissected for the examination of reproductive system under a dissecting microscope (ZEISS Stemi 2000). Terminology for reproductive system follows Gómez (2001). The basal direction starts from the reproductive opening while that of verge starts from the epiphallus following Hwang et al. (2018).

Approximately 30 mg of the foot muscle was used for DNA extraction. The foot muscle was bathed in sterile water for 3–6 hours to remove residual alcohol. Genomic DNA was isolated using Qiagen DNeasy Blood & Tissue kit (Qiagen, Beijing), examined by agarose gel electrophoresis and ultra-micro spectrophotometer (Implen NP80, Germany), then stored at -20 °C for further use. The partial mitochondrial cytochrome c oxidase subunit 1 (COI) was amplified by PCR using apt primer pairs, reaction system, and amplification condition listed in Table 1. The PCR products were analyzed using 1.2% agarose gel electrophoresis.

After sequencing, raw sequences were proof-read on chromatograms and aligned into contigs using BioEdit 7.2 (Hall 1999). Sequence alignments were generated using ClustalW implemented in MEGA6 (Tamura et al. 2013). A total of 35 sequences were used in this study, 23 sequences of which were newly generated and deposited in GenBank (Table 2), and the remainder referenced in Wu et al. (2008), Ding et al. (2016), Ai et al. (2016), and Hu et al. (2019). Pairwise *p*-distances between taxa were calculated using MEGA6 (Tamura et al. 2013) and were compared with the currently known intra and inter- specific differentiation values (p-distances) of Camaenidae (Criscione and Köhler 2014; Ai et al. 2016; Ding et al. 2016). Neighbor-Joining and Minimum-Evolution analyses based on COI sequences were performed using MEGA6 (Tamura et al. 2013). *Amphidromus atricallosus* (Gould, 1843) (Camaenidae) was used

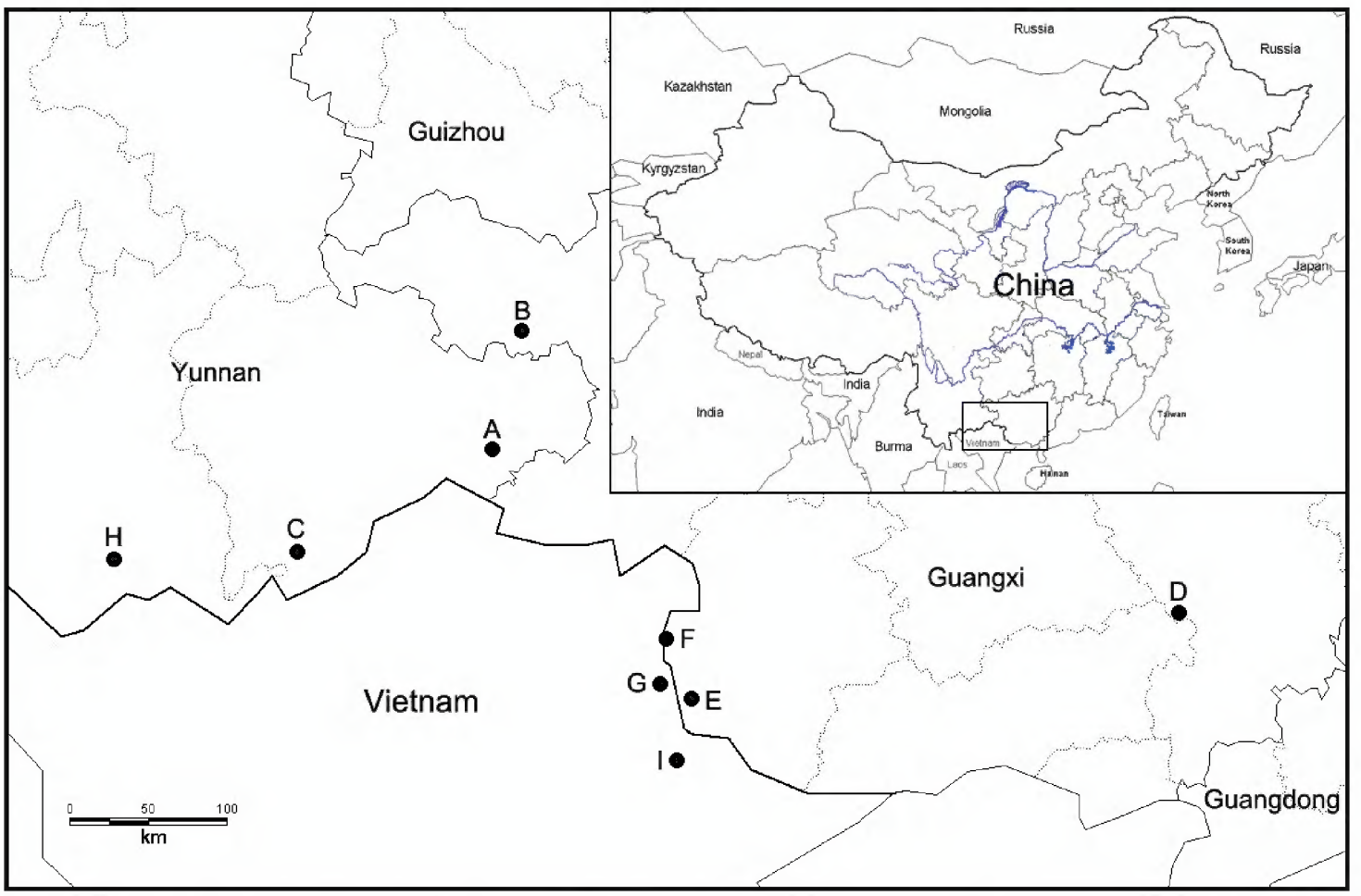

Figure 1. Map of locations of *Camaena* species. *C. funingensis* sp. nov. **A** Laolida, Funing, Wenshan, Yunnan, China. *C. gaolongensis* sp. nov. **B** Dayao, Gaolong, Tianlin, Guangxi, China. *C. maguanensis* sp. nov. **C** Huazhige, Maguan, Wenshan, Yunnan, China. *C. yulinensis* sp. nov. **D** Longquan cave, Yulin, Guangxi, China. *C. vorvonga* **E** Pingxiang, Guangxi, China **F** Longzhou, Guangxi, China **G** That-khe, Vietnam (Type locality). *C. jinpingensis* **H** Jinping, Yunnan, China. *C. longsonensis* **I** Lang-Son, Vietnam.

Table 1. Primer pairs and PCR conditions used in the analyses of the COI gene of *Camaena*.

Gene	COI
Primer pairs (5'-3')	LCO:GGTCAACAAATCATAAAGATATTGG
	HCO:TAAACTTCAGGGTGACCAAAAAATCA
Reaction systems	25 μl Taq PCR MasterMix × 2; 1 μl each primer; 2 μl DNA; 16 μl ddH_2O
Cycling conditions	94 °C: 30 s; 94 °C: 10 s, 45 °C: 50 s, 72 °C: 1 min, 40 cycles; 72 °C: 10 min.
Reference	Folmer et al. 1994

as outgroup. The node support values were assessed by bootstrap resampling using 1000 replicates (Felsenstein 1985).

Abbreviations used in this work:

AG albumen gland;
AH aperture height;
AW aperture width;
BC bursa copulatrix;
COI cytochrome c oxidase subunit 1gene;
E epiphallus;
F flagellum;
FJIQBC Original Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian, China;
GACC General Administration of Customs, People's Republic of China;

HD	hermaphroditic duct;	**PBC**	pedunculus of bursa copulatrix;
ME	Minimum-Evolution;	**PR**	penis retractor muscle;
MNHN	Muséum national d'Histoire naturelle, Paris, France;	**SH**	shell height;
		SW	shell width;
NJ	Neighbor-Joining;	**V**	verge;
O	oviduct;	**Va**	vagina;
P	penis;	**VD**	vas deferens.

Results

Molecular analysis

In this study, a total of 35 sequences of COI from 28 species were used, including eleven sequences from *C. funingensis* sp. nov., *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov., and *C. yulinensis* sp. nov., 8 sequences from sinistral *Camaena* (*C. cicatricosa*, *C. obtecta*, *C. inflata*, *C. connectens*, *C. hahni*, *C. detianensis*, *C. lingyunensis*, *C. poyuensis*), 16 sequences from dextral *Camaena* and one outgroup (*A. atricallosus* Family Camaenidae) listed in Table 2.

Table 2. Sampling GenBank accession numbers used in phylogenetic analysis.

Species	COI accession numbers	References
Camaena funingensis sp. nov.	MT449465, MT449466, MT449467	Present study
Camaena gaolongensis sp. nov.	MT449468, MT449469, MT449470	Present study
Camaena maguanensis sp. nov.	MT449471, MT449472	Present study
Camaena yulinensis sp. nov.	MT449473, MT449474, MT449475	Present study
Camaena vorvonga	MT984239	Present study
Camaena xanthoderma	MT984235	Present study
Camaena xanthoderma polyzona	MT984236	Present study
Camaena hainanensis	MT984234	Present study
Camaena choboensis	MT984240	Present study
Camaena gabriellae	MT984241	Present study
Camaena gabriellae platytaenia	MT984242	Present study
Camaena longsonensis	EF057379	Wu et al. 2008
Camaena jinpingensis	KU586503	Ding et al. 2016
Camaena menglunensis	KU586506	Ding et al. 2016
Camaena inflata	KU586524	Ding et al. 2016
Camaena obtecta	KU055610	Ding et al. 2016
Camaena hahni	KX621263	Ai et al. 2016
Camaena connectens	KU586518	Ding et al. 2016
Camaena poyuensis	KU061273	Ding et al. 2016
Camaena lingyunensis	KX345077	Ai et al. 2016
Camaena cicatricosa	KU061276	Ding et al. 2016
Camaena detianensis	KX345074	Ai et al. 2016
Camaenella platyodon	MH362759	Hu et al. 2019
Camaena leonhardti	MT984237	Present study
Camaena vulpis	MT984238	Present study
Camaena hemiclista	MT984243	Present study
Camaena haematozona	MT984244	Present study
Amphidromus atricallosus	MT984245	Present study

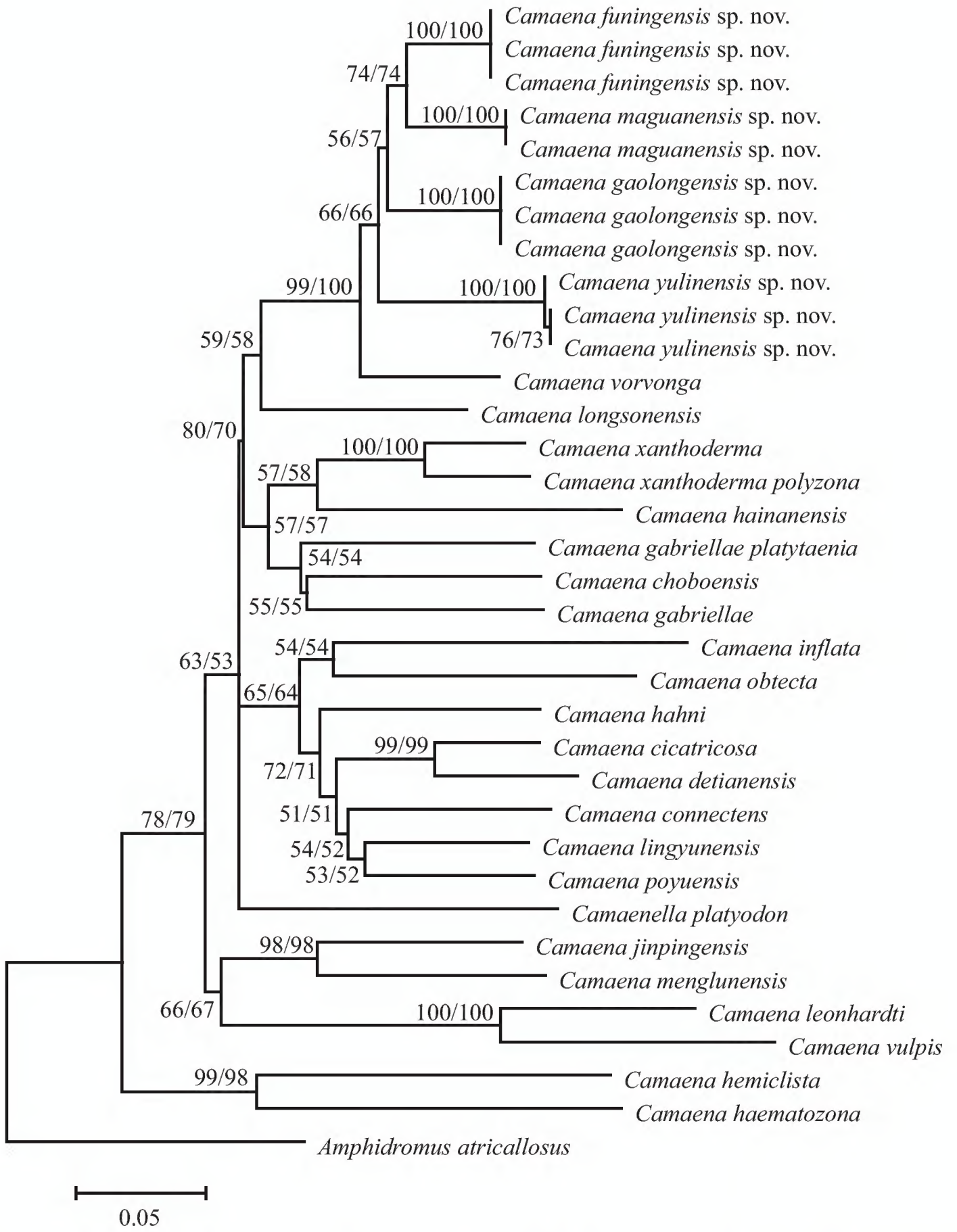

Figure 2. Neighbor-Joining and Minimum-Evolution trees based on analysis of the COI sequences. Numbers beside nodes indicate bootstrapping support (%) for the main clades, based on 1000 replicates.

Inter and intra-specific *P*-distances from COI gene of seven species were calculated and are listed in Table 3. According to the results of the target gene COI, the *p*-distances between *C. funingensis* sp. nov., *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov., and *C. yulinensis* sp. nov. and other dextral *Camaena* were 0.068–0.200, 0.075–0.203, 0.068–0.198 and 0.092–0.202 respectively.

Table 3. Inter and intra-specific *P*-distances of the COI sequences on dextral *Camaena* species.

Sampling	P-distances	
	Within	Between
Camaena funingensis sp. nov.	0.000	0.068–0.200
Camaena gaolongensis sp. nov.	0.000	0.075–0.203
Camaena maguanensis sp. nov.	0.000	0.068–0.198
Camaena yulinensis sp. nov.	0.000–0.002	0.092–0.202
Camaena vorvonga	0.000–0.002	0.089–0.209
Camaena jinpingensis	0.000–0.002	0.196–0.209
Camaena longsonensis	0.000	0.153–0.211

For phylogenetic analysis, results showed that Neighbor-Joining and Minimum-Evolution trees had mostly the same topological structure (Fig. 2), and indicated that phylogenic analyses were relatively correct and reliable. The bootstrap support of each species exceeds 50%. The sinistral camaenids were clearly clustered together. The four dextral new species have the closest phylogenetic relationship to each other and are sister species with *C. vorvonga*. From the tree structure, branch length and comparison of the known species, the phylogenetic trees supported *C. funingensis* sp. nov., *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov., and *C. yulinensis* sp. nov. as new species. Moreover, the four new species all had a closer genetic relationship with each other than with any other *Camaena* species studied here.

Systematics

Camaenidae Pilsbry, 1895

Camaena Albers, 1850

Type species. *Helix cicatricosa* Müller, 1774, subsequent designation by Martens 1860.

Camaena funingensis Zhou, Wang & Lin, sp. nov.

http://zoobank.org/E94E735E-BAD1-4D8C-AC91-5D50DF90AFE5
Figures 3A, 4, 5A, 6, Tables 3–5

Type material. ***Holotype.*** [FJIQBC 19340] Shell height 21.0 mm, shell width 41.0 mm, height of aperture 14.0 mm, width of aperture 18.7 mm, 22 October 2014, collected from the type locality.

Paratype. [FJIQBC 19341–19343] 3 live specimens: 2 adults, 1 juvenile.

Type locality. Laolida, Funing, Wenshan, Yunnan, China (23°31'48.88"N, 105°32'59.70"E).

Etymology. The name of the new species refers to the type locality.

Diagnosis. ***Shell.*** Shell dextral, large, thin, fragile and lucent, low, and flat conical. 4.5 whorls, the front whorls increasing slowly. Spire relatively low. Body whorl rapidly

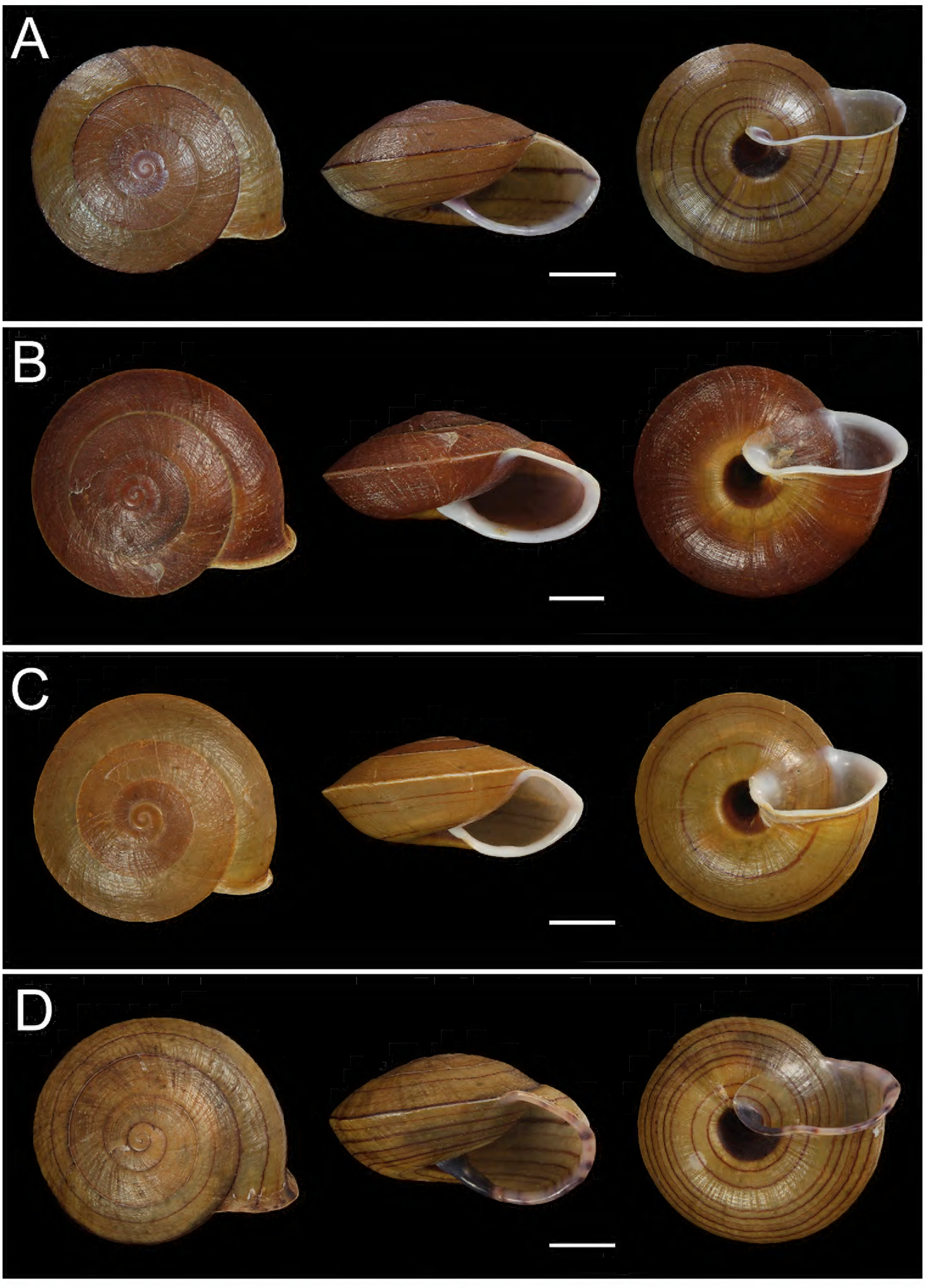

Figure 3. Photographs of the four new species **A** *Camaena funingensis* sp. nov. (holotype, FJIQBC 19340, Laolida, Funing, Yunnan, China) **B** *Camaena gaolongensis* sp. nov. (holotype, FJIQBC 19353, Dayao, Gaolong, Guangxi, China) **C** *Camaena maguanensis* sp. nov. (FJIQBC 19405, Huazhige, Maguan, Yunnan, China) **D** *Camaena yulinensis* sp. nov. (FJIQBC 19460, Longquan cave, Yulin, Guangxi, China). Scale bars: 10 mm.

expanded. Shell light yellowish brown with clear growth lines and spiral bands on the surface. Apex quite blunt. Suture shallow. The protoconch surface smooth, and some short clear growth lines near the inner side of suture under 32 × stereomicroscope. Body whorl with carinate periphery, and a thin reddish brown band on the carina and several sparse bands below the carina. Aperture lunate, slightly descending. Peristome reflected, white, thin, sharp. Columellar lip reflected. Umbilicus reddish brown, large, only 1/5 covered. Inner lip attached to the body whorl, forming translucent callus.

Soft body. Yellowish brown with irregular black lines and spots. Tentacles dark.

Reproductive system. Bursa copulatrix oval and large with long and tapering pedunculus, expanded at the base. Flagellum long, tapering distally. Vas deferens short and thin. Epiphallus long, slightly thick. Penis retractor muscle medium length and slender, becoming wider at the end. Penis swollen and long, with longitudinal, slightly corrugated, strong and widely spaced pilasters internally. Verge ovate, opened terminally, and one clear crack on the verge surface extending from the terminal to the base.

Habitat. The species was found on limestone.

Distribution. Only known from the type locality.

Remarks. *Camaena funingensis* sp. nov. is characterized by a more oblate shape, lower spire, thin and fragile shell, and yellowish brown coloration, which are clearly different from the other dextral camaenids except *C. longsonensis* (Morlet, 1891), *C jinpingensis* Chen, Zhang & Li, 1990, and *C. vorvonga* (Bavay & Dautzenberg, 1900) (Chen et al. 1990; Schileyko 2011). The shells of the above four species are distinct from *C. funingensis* in the following ways:

(1) The umbilicus of *C. funingensis* sp. nov. is only 1/5 covered, while that of *C. longsonensis* is almost covered by reflected columellar lip leaving only a narrow slit, and that of *C. jinpingensis* is fully covered.
(2) *C. funingensis* sp. nov. has several reddish brown bands at the bottom of the body whorl in addition to those on the carina, while only one thin reddish brown band is present on the carinate periphery of *C. vorvonga*.
(3) For *C. funingensis* sp. nov., the verge is ovate and has one clear crack on the surface extending to the base, which makes it stand out other dextral camaenids.

Camaena gaolongensis sp. nov. is distinguishable from *C. funingensis* sp. nov. in having no spiral band. For *C. maguanensis* sp. nov., there is no band on the carinate periphery of the body whorl except for several below the carina. Moreover, the verge of *C. maguanensis* sp. nov. is small and circular. *Camaena yulinensis* sp. nov. differs to *C. funingensis* sp. nov. in having a conical verge and flesh-colored peristome.

P-distances of the COI gene between *C. funingensis* sp. nov. and the other camaenids are 0.068–0.200 (Table 3), and those between *C. funingensis* sp. nov. and *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov. and *C. yulinensis* sp. nov. are 0.075, 0.068 and 0.094 respectively. All of these *P*-distances exceed the maximum intra-specific value 0.059 in the family Camaenidae. On the phylogenetic tree, these four new species are adjacent, hence it is reasonable to designate this as a new species.

***Camaena gaolongensis* Zhou, Wang & Lin, sp. nov.**
http://zoobank.org/1B657A19-59B9-46D2-B874-9DB7120730E9
Figures 3B, 4, 5B, 7, Tables 3–5

Type material. ***Holotype.*** [FJIQBC 19353] Shell height 23.8 mm, shell width 49.0 mm, height of aperture 14.0 mm, width of aperture 19.2 mm, 11 April 2015, collected from the type locality.

Paratype. [FJIQBC 19354] 1 live juvenile, 20 October 2014; [FJIQBC 19355–19356] 2 live adults, 11 April 2015.

Type locality. Dayao, Gaolong, Tianlin, Guangxi, China (24°11'52.33"N, 105°43'40.56"E).

Etymology. The name of the new species refers to the type locality.

Diagnosis. ***Shell.*** Shell dextral, large, thick, strong, low, and flat conical. 4.5 whorls, the front whorls increasing slowly. Spire relatively low. Body whorl rapidly expanded. Shell dark brown with clear and dense growth lines on the surface. Apex quite blunt. Suture shallow. The protoconch surface smooth with scale marks, and some short growth lines clear near the outer side of suture under 32 × stereomicroscope. Body whorl with acute and carinate periphery, but no spiral band. Aperture U-shaped. Peristome reflected, white and thick. Columellar lip reflected. Umbilicus reddish brown, open, large, and only 2/5 covered. Inner lip attached to the body whorl, forming translucent callus.

Soft body. Brown with irregularly black lines and spots. Tentacles dark.

Reproductive system. Bursa copulatrix oval and medium sized with long pedunculus, expanded at the base, becoming thinner at the distal end. Flagellum long and smooth, tapering distally. Vas deferens long and thin. Epiphallus medium length and thick. Penis retractor muscle short, slender basally but wide and flat distally. Penis thick and medium length. Inner penial wall supporting longitudinal, stronger, and more widely spaced pilasters, smooth basally, curved distally. Verge irregularly conical, opened basally, extending from the base to the end, with several slanted wrinkles on the surface.

Habitat. It is common in primary forest and loess areas, but it has not been found on the reclaimed lands outside the primary forest.

Distribution. Only known from the type locality.

Remarks. *Camaena gaolongensis* sp. nov. is clearly different from other dextral camaenids by its quite thick, low, flat, and dark brown conical shell resembling a flying saucer (Chen et al. 1990, Schileyko 2011). Additionally, the longitudinal pilasters on the inner penial wall are stronger and more widely spaced, as well as smooth at the base but curved at the end, which are also distinct from the other dissected *Camaena* snails (Ding et al. 2016, Ai et al.2016).

P-distances of the COI gene between *C. gaolongensis* sp. nov. and other dextral *Camaena* species are 0.075–0.203 (Table 3), and those between *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov., and *C. yulinensis* sp. nov. are 0.085 and 0.104 respectively. Combining the topological structure of the phylogenetic tree, the new species *C. gaolongensis* sp. nov. is distinct from other dextral *Camaena* species.

Figure 4. Photographs of three camaenids **A** *Camaena vorvonga* (Pingxiang, Guangxi, China) **B** *Camaena jinpingensis* (Jinping, Yunnan, China) **C** *Camaena longsonensis* (Lang-Son, Vietnam). Scale bars: 10 mm.

***Camaena maguanensis* Zhou, Wang & Hu, sp. nov.**

http://zoobank.org/EC5431C5-CFB6-4309-80C1-0CF8F3C9BE0E

Figures 3C, 4, 5C, 8, Tables 3–5

Type material. ***Holotype.*** [FJIQBC 19405] Shell height 19.2 mm, shell width 39.0 mm, height of aperture 12.0 mm, width of aperture 16.5 mm, 16 April 2015, collected from the type locality.

Paratype. [FJIQBC 19406] 1 live adult; [FJIQBC 19407–19413] 7 empty shells: 5 adults, 2 juveniles.

Type locality. Huazhige, Maguan, Wenshan, Yunnan, China (22°57'24.48"N, 104°21'12.96"E).

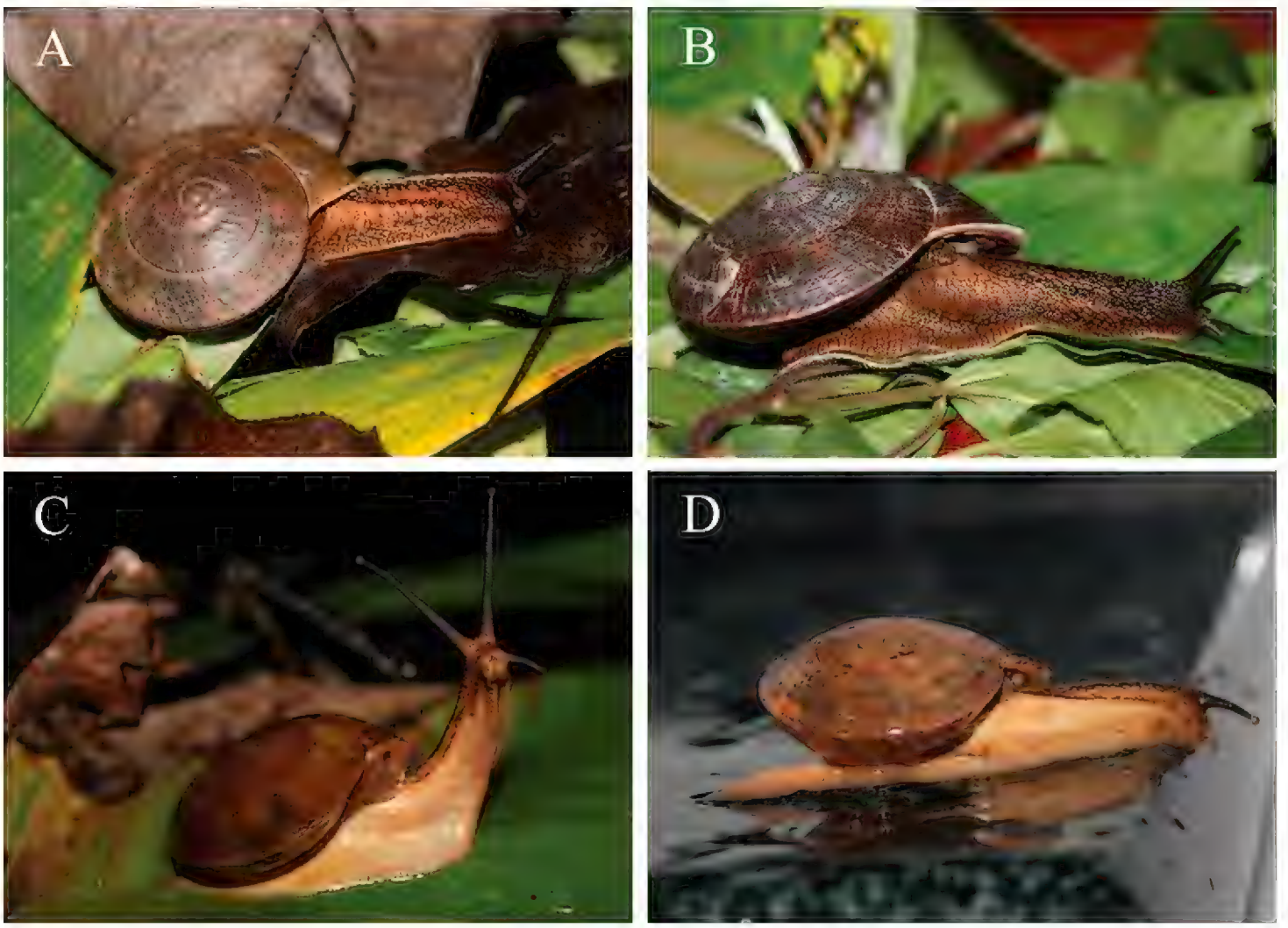

Figure 5. Ecological photographs of snails **A** *Camaena funingensis* sp. nov. (Laolida, Funing, Yunnan, China) **B** *Camaena gaolongensis* sp. nov. (Dayao, Gaolong, Guangxi, China) **C** *Camaena maguanensis* sp. nov. (Huazhige, Maguan, Yunnan, China) **D** *Camaena yulinensis* sp. nov. (Longquan cave, Yulin, Guangxi, China).

Table 4. Adult shell dimensions (mm).

Species	*C. funingensis* sp. nov.	*C. gaolongensis* sp. nov.	*C. maguanensis* sp. nov.	*C. yulinensis* sp. nov.
Voucher	FJIQBC19340–19342	FJIQBC19353 FJIQBC19355–19356	FJIQBC19405–19411	FJIQBC19460–19466 FJIQBC19468–19470
Sample size	3	3	7	10
SH	19.5–21.0 (20.17±0.62)	23.5–24.5 (23.93±0.42)	19.2–22.0 (20.36±0.90)	19.8–23.0 (21.35±1.05)
SW	39.2–41.0 (40.23±0.76)	47.0–50.0 (48.67±1.25)	38.0–40.5 (39.24±0.74)	37.0–42.6 (40.54±1.58)
SW/SH	1.95–2.03 (2.00±0.03)	2.00–2.06 (2.03±0.02)	1.84–2.03 (1.93±0.06)	1.84–1.96 (1.90±0.03)
AH	13.4–14.0 (13.63±0.26)	13.8–14.2 (14.00±0.16)	12.0–13.1 (12.64±0.34)	13.0–14.6 (13.76±0.48)
AW	18.0–18.7 (18.30±0.29)	19.0–19.4 (19.20±0.16)	16.5–18.1 (17.22±0.56)	17.5–21.6 (19.11±1.46)
AW/AH	1.34–1.35 (1.34±0.01)	1.37–1.38 (1.37±0.00)	1.33–1.39 (1.36±0.02)	1.33–1.48 (1.39±0.06)

Etymology. The name of the new species refers to the type locality.

Diagnosis. ***Shell.*** Shell dextral, large, thin, fragile, and glossy, low and flat conical. 4.5 whorls, the front whorls increasing slowly. Spire relatively low. Body whorl rapidly expanded. Shell yellowish with unclear growth lines and spiral bands on the surface.

Apex quite blunt. Suture shallow. The protoconch surface smooth, some short growth lines visible near the two sides of suture under 32 × stereomicroscope. Last whorl with quite acute carina at periphery and a shallow groove-like depression above and below the carina. No band on the carina, but several reddish brown and sparse spire bands below the carina. Aperture crescent-shaped. Peristome reflected, white and thick. Columellar lip reflected. Umbilicus reddish brown, open, large and only 2/5 covered. Inner lip attached to the body whorl, forming translucent callus.

Soft body. Light yellowish brown with black lines. Tentacles dark.

Reproductive system. Bursa copulatrix oval, small, with quite long and tapering pedunculus. Flagellum long, tapering distally. Vas deferens long and thin. Epiphallus medium thickness and length. Penis retractor muscle very short and slender. Penis long with a short protrusion at the middle. Inner penial wall with longitudinal, slightly straight and smooth pilasters. Verge circular, somewhat small, opened basally, extending from the base to the end.

Habitat. The species was found on limestone in Maguan county of Yunnan province, China.

Distribution. Only known from the type locality.

Remarks. *Camaena maguanensis* sp. nov. is clearly different from other dextral camaenids with a lower conical shell. In particular, *C. maguanensis* sp. nov. has a large and open umbilicus, which distinguishes it from *C. longsonensis* and *C. jinpingensis*. Although the umbilicus of *C. maguanensis* sp. nov. is similar to that of *C. vorvonga*, some differences are obvious. For example, *C. maguanensis* sp. nov. has no spiral band on the carinate periphery of the body whorl but some spaced bands at the base. The shell of *C. maguanensis* sp. nov. is yellowish, but that of *C. gaolongensis* sp. nov. is dark brown. On the other hand, *C. maguanensis* sp. nov. has a circular and slightly smaller verge.

P-distances of the COI gene between this new species and the other dextral species are 0.068–0.198 (Table 3), and that between *C. maguanensis* sp. nov. and *C. yulinensis* sp. nov. is 0.108, also exceeding 0.059 (currently the maximum differentiation value (p-distance) of Camaenidae) (Criscione and Köhler 2014), and the topology of the phylogenetic tree also supports the new species.

Camaena yulinensis Zhou, Wang & Hu, sp. nov.

http://zoobank.org/3038DBDB-A3B2-4364-B2D3-CB7E694EA8ED

Figures 3D, 4, 5D, 9, Tables 3–5

Type material. ***Holotype.*** [FJIQBC 19460] Shell height 21.0 mm, shell width 40.5 mm, height of aperture 13.5 mm, width of aperture 18.2 mm, 21 September 2014, collected from the type locality.

Paratype. [FJIQBC 19461–19466] 6 specimens: 3 live adults, 3 empty adult shells, 4 November 2013; [FJIQBC 19468–19472] 5 specimens: 3 live adults, 2 empty juvenile shells, 21 September 2014.

Type locality. Longquan cave, Yulin, Guangxi, China (22°36'41.24"N, 109°45'21.36"E).

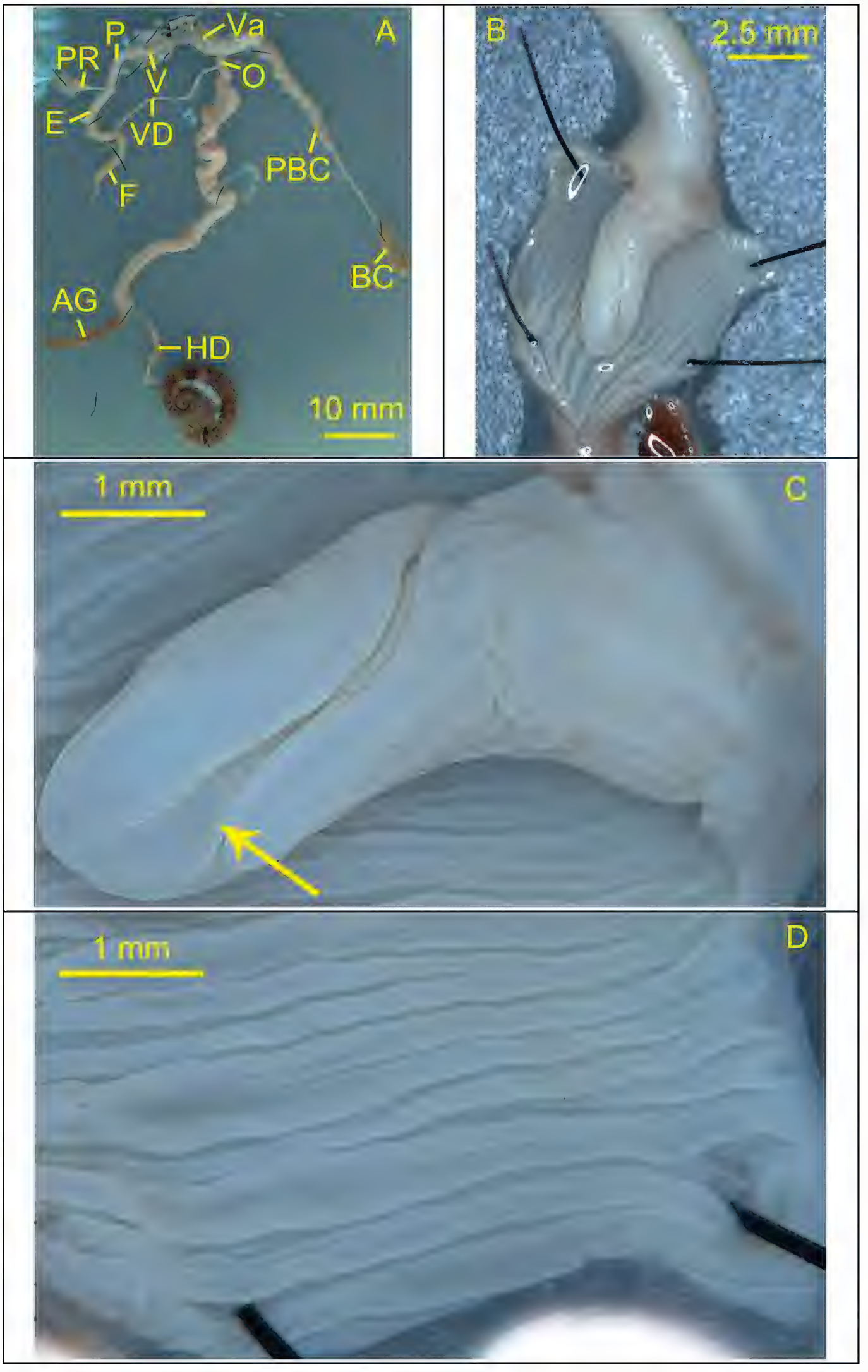

Figure 6. Reproductive system of the snail *Camaena funingensis* sp. nov. (holotype, FJIQBC 19340, Laolida, Funing, Yunnan, China) **A** reproductive organ **B** penis **C** verge **D** inner penial wall. The arrow indicates opening position of the verge.

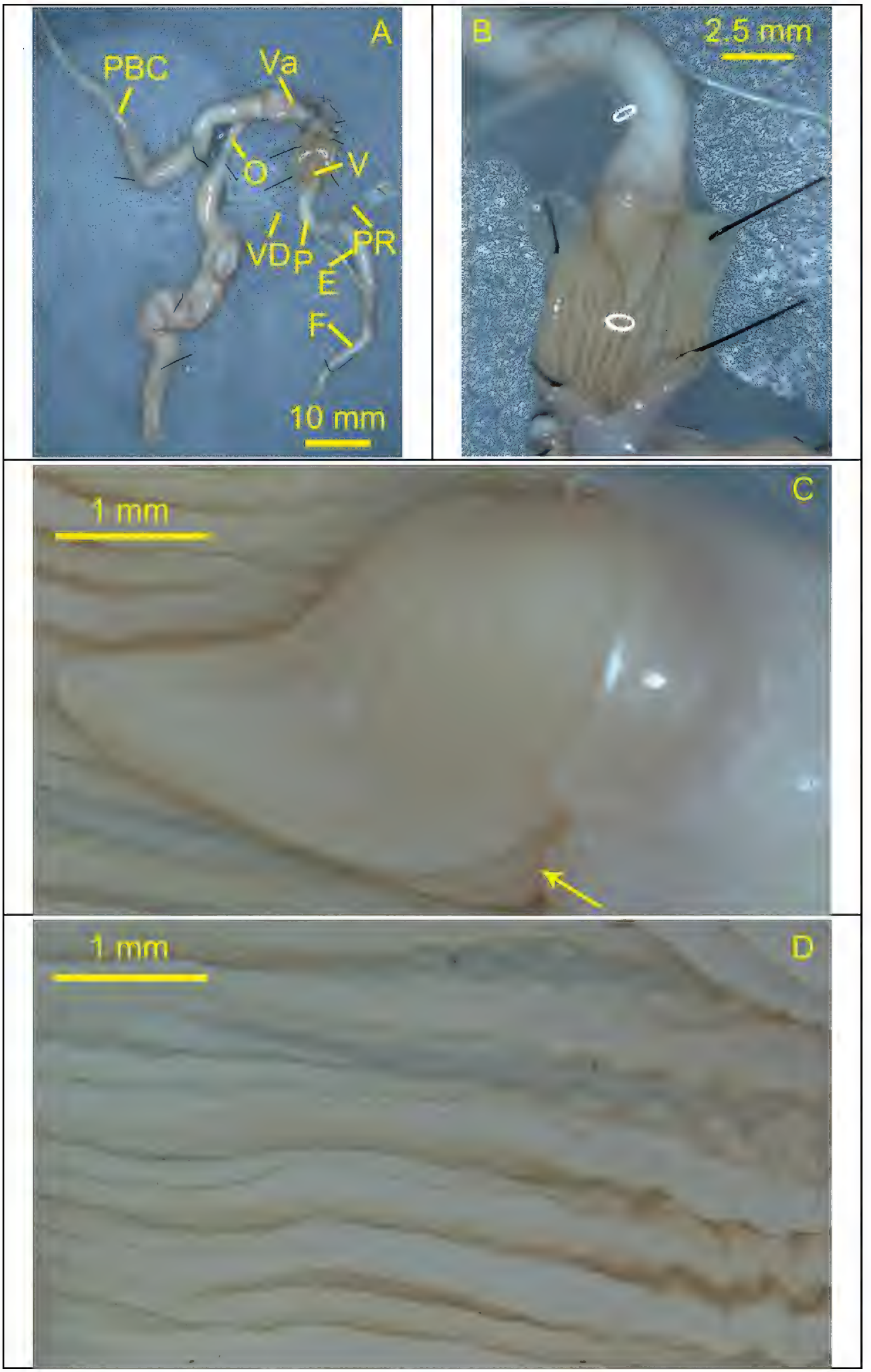

Figure 7. Reproductive system of the snail *Camaena gaolongensis* sp. nov. (holotype, FJIQBC 19353, Dayao, Gaolong, Guangxi, China) **A** reproductive organ **B** penis **C** verge **D** inner penial wall. The arrow indicates opening position of the verge.

Figure 8. Reproductive system of the snail *Camaena maguanensis* sp. nov. (FJIQBC 19405, Huazhige, Maguan, Yunnan, China) **A** reproductive organ **B** penis **C** verge **D** inner penial wall. The arrow indicates opening position of the verge.

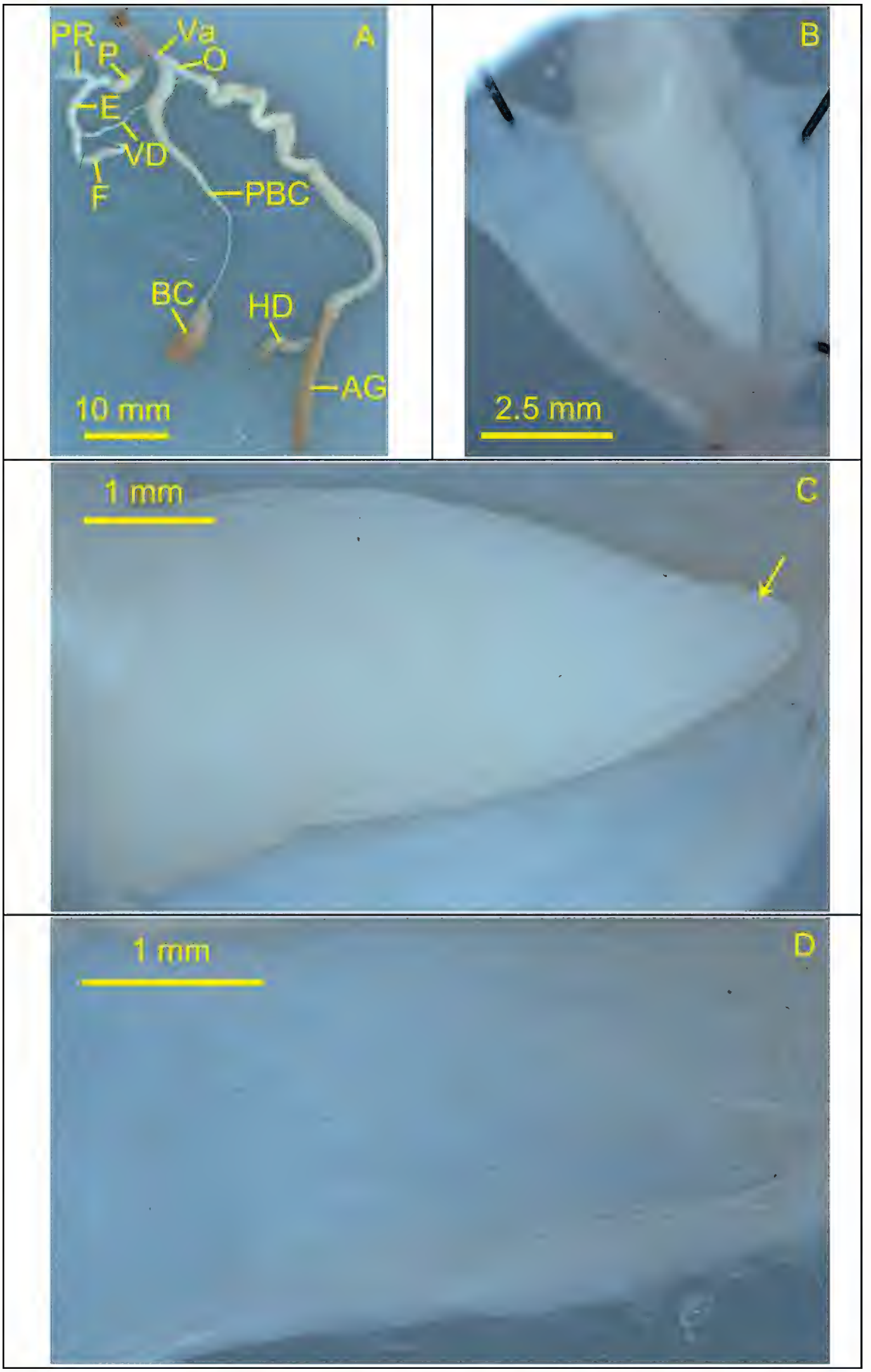

Figure 9. Reproductive system of the snail *Camaena yulinensis* sp. nov. (FJIQBC 19460, Longquan cave, Yulin, Guangxi, China) **A** reproductive organ **B** penis **C** verge **D** inner penial wall. The arrow indicates opening position of the verge.

Table 5. Diagnostic comparisons of morphological characters of the four new species.

Character	*C. funingensis* sp. nov.	*C. gaolongensis* sp. nov.	*C. maguanensis* sp. nov.	*C. yulinensis* sp.nov.
Shell thickness	thin	quite thick	thin	thin
Shell color	light yellowish brown	dark brown	yellowish	light yellowish
Periphery	carinate	acute and carinate	acute and carinate	carinate
Growth lines	clear	clear and dense	unclear	clear and dense
Umbilicus	only 1/5 covered	only 2/5 covered	only 2/5 covered	1/3 covered
Verge	ovate	short conic	circular and small	long conic
Verge opening	terminally, one clear crack on the surface extending from the end to the base	basally, one crack on the side surface extending from the base to the end	basally, one crack on the surface extending from the base to the end	terminally

Etymology. The name of the new species refers to the type locality.

Diagnosis. ***Shell.*** Shell dextral, large, thin, fragile, and slightly lucent, low and flat conical. 4.5 whorls, the front whorls increasing slowly. Spire relatively low. Body whorl rapidly expanded. Shell light yellowish with clear and dense growth lines and spiral bands on the surface. Apex quite blunt. Suture shallow. The protoconch surface smooth for most individuals, but a few are rough. Growth lines clear near the outer side of suture under 32 × stereomicroscope. Last whorl with carinate periphery, a thin reddish brown spiral band on the carina, and many reddish brown spiral bands of different thickness on the upper and lower parts. Aperture lunate. Peristome reflected, flesh-colored, thin, sharp. Columellar lip reflected. Umbilicus reddish brown, open, large, and only 1/3 covered. Inner lip attached to body whorl, forming translucent callus.

Soft body. Pale yellow with irregular black lines. Tentacles dark brown.

Reproductive system. Bursa copulatrix oval, large, with long and tapering pedunculus. Flagellum long and slightly thick, tapering distally. Vas deferens short and thin. Epiphallus medium length and slightly thick. Penis retractor muscle short and wide. Penis short and swollen at distal 1/3, with longitudinal, thin, smooth pilasters internally. Verge conical, large, opened terminally, with some irregular wrinkles on the surface.

Habitat. The species was found on limestone in Yulin city, Guangxi province.

Distribution. Only known from the type locality.

Remarks. *Camaena yulinensis* sp. nov. differs from *C. longsonensis* and *C. jinpingensis* in the key characteristic of large open umbilicus. This new species not only has spiral bands with different thickness on the body whorl but also has a flesh-colored peristome compared to *C. vorvonga*. The differences between this species and the other three new *Camaena* species herein have already been described above.

P-distances of the COI gene between *C. yulinensis* sp. nov. and the other dextral congeners ranges from 0.092 to 0.202 (Table 3) and the phylogenetic topology tree supports the establishment of this new species.

Discussion

We describe four new species of dextral *Camaena* snails, namely *C. funingensis* sp. nov., *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov. and *C. yulinensis* sp. nov., which

are distinguished from their congeners by their shell morphologies, especially the low and flat shell shape, the large open umbilicus, the acute and carinate periphery of the body whorl, as well as features in their reproductive systems and molecular characteristics. Among the first three new species, the differences of shells and genitals are obvious. Although *C. funingensis* sp. nov. and *C. yulinensis* sp. nov. are similar in shell morphology except size, color and umbilicus, the former has an ovate and terminally opened verge and one clear crack on the surface extending from the end to the base, as well as strong and widely spaced penis pilasters, that distinguish it from *C. yulinensis* sp. nov. with a conical verge, thin penial inner pilasters and without crack on the surface (Figs 3, 5–9). Nonetheless, the two similar-shaped species are relatively distantly related genetically (Fig. 2).

Some scholars have considered genetic distance as one of the more important pieces of evidence used for identifying new species and revising species; for example, in the Asian camaenids *Luchuhadra* (Kameda et al. 2007) and *Satsuma* (Wu et al. 2008), the Australian camaenid *Kimberleytrachia* (Criscione and Köhler 2014), and *Camaena* (Ai et al. 2016; Ding et al. 2016). In the present study, the *p*-distances between *C. funingensis* sp. nov., *C. gaolongensis* sp. nov., *C. maguanensis* sp. nov., *C. yulinensis* sp. nov., and the other dextral *Camaena* was substantial: 0.068–0.200, 0.075–0.203, 0.068–0.198, and 0.092–0.202 respectively for the mitochondrial COI barcoding region (Table 3). These numbers exceed the intra-specific differentiation values (*p*-distances) of Camaenidae (for *Camaena*, minimum 0.00, maximum 0.018 in Ding et al. (2016), minimum 0.00, maximum 0.019 in Ai et al. (2016), for *Kimberleytrachia*, minimum 0.00, maximum 0.059, mean 0.026 in Criscione and Köhler (2014). Based on these considerations, inter-specific differentiation supports the recognition of the four new species.

In the phylogenetic analyses, *C. vorvonga* and *C. longsonensis*, which were placed in informal subgeneric group I, have a close relationship, while they are distant from *C. jinpingensis* that originally also belonged to group I. In the future, more species and sequences will be needed for a more robust analysis of camaenid phylogeny.

During our long-term field investigations, we observed that most *Camaena* species have a narrow distribution and a low population density, and only inhabit primary forests. An exception to this is *C. cicatricosa*, which is widespread and has high population densities (Ai et al. 2016; Ding et al. 2016). In recent years, with the development of the Chinese economy, areas of primary forest have been decreasing and the habitats of *Camaena* species are becoming increasingly restricted and threatened. Therefore, it is necessary to maximize forest protection, prevent deforestation, and prevent excessive tourist development to preserve the biodiversity of these terrestrial mollusks and other animals and plants.

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References

Adams H (1870) Descriptions of a new genus, and of eighteen new species of Molluscs. Proceedings of the Zoological Society of London 1870: 5–9.

Albers JC (1850) Die Heliceen, Nach Natürlicher Verwandtschaft Systematisch Geordnet. T. C. F. Enslin, Berlin, 262 pp. https://www.biodiversitylibrary.org/page/11965983

Ai HM, Lin JH, Wang P, Zhou WC, Hwang CC (2016) Descriptions of two new species of the genus *Camaena* from Guangxi, China (Gastropoda, Stylommatophora, Camaenidae). ZooKeys 634: 29–45. https://doi.org/10.3897/zookeys.634.10236

Bavay A, Dautzenberg P (1900) Diagnose de nouveaux mollusques d'Indo-Chine. Journal de Conchyliologie 48: 108–122.

Bavay A, Dautzenberg P (1909) Description de coquilles nouvelles de l'Indo-Chine. Journal de Conchyliologie 57: 163–206.

Chen DN, Gao JX (1987) Economic Fauna Sinica of China (Terrestrial Mollusca). Science Press, Beijing, 186 pp.

Chen DN, Zhang GQ (1999) Studies on the genus *Camaena* from China, with description of two new species (Gastropoda: Camaenidae). Transactions of the Chinese Society of Malacology 8: 28–43.

Chen DN, Zhang QG, Li BH (1990) A new terrestrial snail species from China. Zoological Systematics 2(6): 33–35.

Criscione F, Köhler F (2014) Molecular phylogenetics and comparative anatomy of *Kimberleytrachia* Köhler, 2011 – a genus of land snail endemic to the coastal Kimberley, Western Australia with description of new taxa (Gastropoda, Camaenidae). Contributions to Zoology 83(4): 245–267. https://doi.org/10.1163/18759866-08304003

Dautzenberg PH, Fischer H (1906) Liste de mollusques récoltés par M. H. Mansuy en Indochine et au Yunnan, et description d'espèces nouvelles. Journal de Conchyliologie, Paris 53: 343–471.

Dillon RT (1984) What shall I measure on my snails? Allozyme data and multivariate analysis used to reduce the nongenetic component of morphological variance in *Goniobasis proxima*. Malacologia 25: 503–511.

Ding HL, Wang P, Qian ZX, Lin JH, Zhou WC, Hwang CC, Ai HM (2016) Revision of sinistral land snails of the genus *Camaena* (Stylommatophora, Camaenidae) from China based on morphological and molecular data, with description of a new species from Guangxi, China. ZooKeys 584: 25–48. https://doi.org/10.3897/zookeys.584.7173

Eddy S (1998) Profile hidden Markov models. Bioinformatics 14: 755–763. https://doi.org/10.1093/bioinformatics/14.9.755

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.2307/2408678

Fischer H (1898) Notes sur la faune du Haut Tonkin. III. Liste des mollusques recueillis par le Dr A. Billet. Bulletin scientifique de la France et de la Belgique 28: 310–338.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gómez BJ (2001) Structure and functioning of the reproductive system. In: Baker GM (Ed.) The Biology of Terrestrial Molluscs. CABI Publishing, Oxon, 307–330. https://doi.org/10.1079/9780851993188.0307

Gould AA (1843) Description of land mollusks from the province of Tavoy, in British Burmah. Proceedings of the Boston Society of Natural History 1843: 137–141.

Gredler V (1888) Zur Conchylien-Fauna von China. XIII. Stück. Jahrbücher der deutschen malakozoologischen Gesellschaft, 14: 343–373.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.1021/bk-1999-0734.ch008

Hoso M, Kameda Y, Wu SP, Asami T, Kato M, Hori M (2010) A speciation gene for left–right reversal in snails results in anti-predator adaptation. Nature Communications 1: e133. https://doi.org/10.1038/ncomms1133

Hu ML, Wang P, Chen Y, Zhang MZ, Yang SP, Lin JH, Zhou WC (2019) The mitochondrial genome of the land snail *Camaenella platyodon* (Pfeiffer, 1846) (Stylommatophora, Camaenidae), Mitochondrial DNA Part B 4: 2753–2754. https://doi.org/10.1080/23802359.2019.1644559

Hwang CC (2012) Anatomy and taxonomy of *Satsuma succincta* (Adams, 1866) and *Satsuma batanica pancala* (Schmacker & Boettger, 1891) (Gastropoda: Camaenidae) from southern Taiwan. Bulletin of Malacology 35: 1–11.

Hwang CC, Okuba K, Tada A (2018) *Satsuma jinlunensis*– a new species from Taiwan (Stylommatophora: Camaenidae). Molluscan Research 38: 1–6. https://doi.org/10.1080/13235818.2017.1358340

Inkhavilay K, Sutcharit C, Bantaowong U, Chanabun R, Siriwut W, Srisonchai R, Polyotha A, Jirapatrasilp P, Panha S (2019) Annotated Checklist of the Terrestrial Molluscs from Laos (Gastropoda: Neritimorpha, Caenogastropoda and Heterobranchia). ZooKeys 834: 1–166. https://doi.org/10.3897/zookeys.834.28800

Kameda Y, Kawakita A, Kato M (2007) Cryptic genetic divergence and associated morphological differentiation in the arboreal land snail *Satsuma* (*Luchuhadra*) *largillierti* (Camaenidae) endemic to the Ryukyu Archipelago, Japan. Molecular Phylogenetics and Evolution 45: 519–533. https://doi.org/10.1016/j.ympev.2007.03.021

Koetschan C, Förster F, Keller A, Schleicher T, Ruderisch B, Schwarz R, Müller T, Wolf M, Schultz J (2010) The ITS2 Database III – sequences and structures for phylogeny. Nucleic Acids Research 38: 275–279. https://doi.org/10.1093/nar/gkp966

Kuroda L, Habe T (1949) Helicacea. Osaka, 129 pp. [1 pl.]

Mabille J (1889) Contribution à la faune malacologique du Tonkin. A. Masson, Meulan, 20 pp.

Minato H (1980) Land snail fauna of Miyako Islands, the southern Ryukyu, Japan. Venus 39: 83–99.

Möllendorff OF von (1882) Diagnoses specierum novarum Chinae meridionalis. Jahrbücher der Deutschen Malakozoologischen Gesellschaft 9: 179–188.

Möllendorff OF von (1885) Materialien zur Fauna von China. Die Auriculaceen. Jahrbücher der Deutschen Malakozoologischen Gesellschaft 12: 349–398.

Möllendorff OF von (1888) Mittheilungen aus dem Gebiete der Malakozoologie. Diagnoses specierum novarum sinensium. Nachrichtsblatt der deutschen malakozoologischen Gesellschaft 20(2/3): 38–44.

Morlet L (1891) Diagnoses Molluscorum novorum, in Indo-Chinâ collectorum. Journal de Conchyliologie, Paris 39: 25–28.

Müller OF (1774) Vermivm terrestrium et fluviatilium, v.2. Havni.apud Heineck et Faber, 214 pp.

Páll-Gergely B, Hunyadi A, Otani JU, Asami T (2016) An impressive new camaenid, *Entadella entadiformis* gen. & sp. n. from Guangxi, China (Gastropoda: Pulmonata). Journal of Conchology 42(4): 167–179.

Palumbi S, Martin A, Romano S, Mcmillan WO, Stice L, Grabowwski G (1991) The Simple Fool's Guide to PCR. Department of Zoology, University of Hawaii, Honolulu, 46 pp.

Pilsbry HA (1893) Preliminary outline of a new classification of the helices. Academy of Natural Sciences of Philadelphia, Philadelphia, 44: 387–404.

Pilsbry HA (1894) Manual of Conchology. Series 2, vol.9. Academy of Natural Sciences, Philadelphia, 302 pp.

Richardson L (1985) Camaenidae: catalog of species. Tryonia 12: 1–479.

Schileyko AA (2003) Treatise on recent terrestrial pulmonate molluscs. Part 11. Trigonochlamydidae, Papillodermidae, Vitrinidae, Limacidae, Bielziidae, Agriolimacidae, Boettgerillidae, Camaenidae. Ruthenica Suppl 2: 1510–1621.

Schileyko AA (2011) Check-list of land pulmonate molluscs of Vietnam (Gastropoda: Stylommatophora). Ruthenica 21(1): 1–68.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Vaught KC (1989) A Classification of the Living Mollusca. Melbourne, 189 pp.

Wall-Palmer D, Hegmann M, Goetze E, Peijnenburg KTCA (2019) Resolving species boundaries in the Atlanta brunnea species group (Gastropoda, Pterotracheoidea). ZooKeys 899: 59–84. https://doi.org/10.3897/zookeys.899.38892

Wu M, Chen ZY, Zhu XR (2019) Two new camaenid land snails (Eupulmonata) from Central China. ZooKeys 861: 129–144. https://doi.org/10.3897/zookeys.861.35430

Wu SP, Hwang CC, Lin YS (2008) Systematic revision of the arboreal snail *Satsuma* albida species complex (Mollusca: Camaenidae) with descriptions of fourteen new species from Taiwan. Zoological Journal of the Linnean Society 154: 437–493. https://doi.org/10.1111/j.1096-3642.2008.00415.x

Yen TC (1939) Die chinesischen Land-und Süsswasser Gastropoden des Natur-Museums Senckenberg. Vittorio Klostermann, Frankfurt-am-Main. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 444: 1–235.

Zilch A (1959–1960) Gastropoda. Teil 2: Euthyneura. Handbuch der Paläozoologie, Band 6. Berlin-Nikolassee, Gebrüder Borntraeger, 834 pp.

Zilch A (1964) Die Typen und Typoid des Natur-Museums Senckenberg, 29: Mollusca, Camaenidae (3). Archiv für Molluskenkunde 93: 243–262.